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BRIEF COMMUNICATION

Human leukocyte antigen class I and II haplotypes and risk of cervical cancer

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Abstract

Human leukocyte antigen (HLA) variations may affect immune response to human papillomavirus infection and subsequent cervical neoplasia risk. We investigated the frequency and relationship between HLA-A-B and HLA-A-B-DR haplotypes among women with cervical cancer/high-grade lesions ($n = 365$) and cytologically normal population controls ($n = 681$) within three cervical neoplasia studies in the US and Costa Rica. Notable differences in haplotype frequencies were observed; the HLA-A*01-B*08 haplotype occurred in >5% of US Caucasians but in <1% of Costa Ricans. The most prevalent HLA-A*24-B*40-DR*04 haplotype in Costa Rica (5%) was found in <1% of US Caucasians. No HLA haplotype was significantly associated with cervical neoplasia, suggesting that individual allele associations reported to date (e.g. HLA-DR*13) are not likely explained by underlying haplotypes.

The polymorphic human leukocyte antigen (HLA) class I (e.g. HLA-A, HLA-B, and HLA-C) and class II (e.g. HLA-DR) molecules play a critical role in host immune response to infection (1). Although specific HLA alleles have been shown to alter cervical cancer risk including protective HLA-DRB*13 and disadvantageous HLA-B*07 (2), there are sparse data regarding associations between HLA haplotypes and cervical cancer. Because HLA-A and HLA-B alleles are in linkage disequilibrium with HLA-DR alleles, it remains possible that allele-specific associations reported to date are either due to the specific allele or due to their linkage disequilibrium with other HLA alleles. To date, emphasis has been placed on studying specific single HLA alleles because of sample size considerations; consequently, potential HLA haplotype associations may have been overlooked. We therefore pooled data across three epidemiological studies composed of women in Costa Rica and the US to determine the prevalence of HLA haplotypes in our populations and subsequently investigate whether those with sufficient prevalence for analysis altered risk of cervical cancer.

Our analytic group consisted of 365 women with cancer or high-grade squamous intraepithelial lesions (HSILs) and 681 with normal cytologic test results iteratively derived to meet case and control specifications from women enrolled in three National Cancer Institute-sponsored studies: (i) a population-based cohort of 10,077 women in Guanacaste, Costa Rica (3), (ii) a cohort of 23,702 women in Portland, OR (4), and (iii) a multicenter study of 529 women with histological subtypes of cervical cancer in the Eastern US (5). HLA class I and II alleles were typed for subsets of both the Costa Rican study (124 cases and 118 controls) and the Eastern US study (117 cases and 213 controls). In the Portland study (124 cases and 350 controls), all HLA class I alleles were typed; HLA-DRB*13 was the only HLA class II allele typed in this study. HLA class I and II loci were molecularly typed using polymerase chain reaction (PCR)/sequence-specific oligonucleotide hybridization primer (SSOP)-based protocols developed by the 13th International Histocompatibility Workshop (6) with DNA extracted from cervicovaginal or blood/buffy coat

specimens collected from each participant. Cervicovaginal samples were PCR-tested for papillomavirus (HPV) DNA as described previously (4, 5, 7). Briefly, in the Portland, Oregon cohort, HPV was typed with MY09/11 consensus primers via dot blot. In the Eastern US case-control study, HPV was also typed with MY09/11 consensus primers, but by strip technology. In the Costa Rican cohort, HPV typing was performed by both PCR (with MY09/11) and hybrid capture tube test.

HLA-A-B and HLA-A-B-DR haplotype frequencies were determined using 'FastHap', which determines haplotypes by estimation maximization (8). Haplotypes occurring in >5% in any one of the three study case or control groups were included in further analyses. In all, seven HLA-A-B and three HLA-A-B-DR haplotypes satisfied our overall prevalence criteria of ≥5% in cases or controls; for these haplotypes, we assigned the inferred haplotype to each individual and performed logistic regression analysis to provide risk estimates adjusted by study to account for potential population differences. We thus calculated the odds ratios (OR) and 95% confidence intervals (CI) (SAS version 8.2, SAS Institute, Cary, NC) for cervical neoplasia to determine the magnitude and statistical significance of associations.

Although we also attempted to identify HLA haplotypes associated with disease progression by conducting HPV-restricted analyses where cancer/HSIL cases were compared with low-grade squamous intraepithelial lesion (LSIL) and HPV+ controls, these data are not shown due to the minimal number of HPV-positive population controls.

Overall haplotype frequencies as estimated by 'FastHap' based on data for each control group yielded over 200 HLA-A-B-DR haplotypes in the Eastern US and Costa Rican control populations, and over 150 HLA-A-B haplotypes in the study populations. As expected, however, the vast majority of haplotypes were estimated to occur in few individuals and in <5% of the population. Interestingly, the most common haplotypes in the US Caucasian populations did not overlap with the most common haplotypes identified in the Costa Rican population. While the most common haplotypes in controls as identified by 'FastHap' included HLA-A*01-B*08-DR*03 and HLA-A*02-B*44-DR*04 in the Eastern US Caucasian controls, the most common haplotypes for Costa Rican controls, an ethnically admixed population, were HLA-A*24-B*40-DR*04 and HLA-A*02-B*40-DR*04 (Table 1). Between the two US-based studies of predominantly Caucasian descent, however, the most prevalent haplotypes were consistent and included HLA-A*01-B*08, HLA-A*02-B*07, HLA-A*02-B*44, and HLA-A*03-B*07 (Table 2). Again, the most common Caucasian HLA-A-B haplotypes were not the same as those in the Costa Rican control population.

For HLA haplotypes with >5% prevalence in either cases or controls, we evaluated their association with cervical cancer or precancer (CIN3) in a pooled analysis; we also included in our evaluation the haplotypes that included previously reported HLA allele–cervical cancer associations, namely HLA-DR*13 and HLA-B*07. In all, we calculated the risk estimates for nine HLA-A-B haplotypes and seven HLA-A-B-DR haplotypes. Of the HLA-A-B haplotypes evaluated, none were statistically significantly associated with risk of cervical neoplasia (Table 3). Of the HLA-A-B-DR haplotypes evaluated, none with ≥5% prevalence were statistically significantly associated with cervical neoplasia. Only one haplotype (HLA-A*02-B*07-C*07) was statistically significantly associated with an increased risk of cervical neoplasia with an OR of 3.9. However, the number of cases and controls was small (nine cases and four controls), and we believe that this association is likely influenced by the HLA-B*07 allele for which previous increases in risk were reported; in addition, due to the low prevalence of this haplotype, we also do not believe that the single haplotypic association could have accounted for all previous HLA-B*07 associations reported to date. Although none of the haplotypes containing HLA-DR*13 were significantly associated with cervical neoplasia, their associations were all <1 and thus consistent with decreases in risk (HLA-A*02-B*15-DR*13, OR = 0.3; HLA-A*02-B*44-DR*13, OR = 0.6; HLA-A*02-B*51-DR*13, OR = 0.4). We believe that these decreases in risk were largely due to the HLA-DR*13 allele as previously reported particularly given the relatively low prevalence of each of these haplotypes. We therefore also conclude that it is unlikely that the HLA-DR*13 associations previously observed are attributable to the underlying haplotype reported here.

We previously published individual HLA class I and II allele associations with cervical cancer (4, 9). In the present analysis, we investigate the association between inferred HLA class I and II haplotypes and cervical cancer risk. The haplotype frequencies for the majority of haplotypes

Table 1 Most frequent HLA-A-B-DR haplotypes in respective study population controls

Eastern US (Caucasians) *n* 213		Costa Rica (Hispanics) *n* 118	
A-B-DR	%	A-B-DR	%
01-08-03	4.9	24-40-04	4.8
02-44-04	3.0	02-40-04	3.9
02-15-13	1.9	02-35-04	2.8
03-07-15	1.9	02-07-15	2.3
02-44-13	1.8	68-40-04	1.6
02-35-01	1.7	24-35-04	1.6
02-57-07	1.7	02-39-04	1.5
03-35-01	1.6	02-51-13	1.5
02-13-07	1.3	24-15-04	1.5
24-15-11	1.1	03-07-15	1.3

Table 2 Most frequent HLA-A-B haplotypes in respective study population controls

Eastern US study controls (Caucasians)		Portland Kaiser controls (Caucasians)		Costa Rica controls (Hispanics)	
A-B	%	A-B	%	A-B	%
01-08	5.4	01-08	6.8	02-35	3.8
02-07	3.5	02-44	5.7	02-07	3.1
03-07	3.1	03-07	5.1	68-40	2.6
01-07	2.5	02-07	4.8	24-40	2.5
02-15	2.4	02-40	3.6	24-40	2.3
02-44	2.2	02-51	3.6	03-07	2.0
02-44	1.9	02-27	2.6	02-15	1.8
03-35	1.8	29-44	2.5	68-53	1.5
02-40	1.7	02-15	2.4	68-39	1.5
68-44	1.6	03-35	2.2	31-35	1.5

within all three studies were largely <5%, and we thus did not pursue further analysis. Our study strengths included extensive HLA genotyping, larger sample size obtained by merging data from three studies, and the ability to identify consistent patterns across them should they exist. Although we adjusted our pooled analysis by study, our study limitations still include the potential for population stratification and small sample size per individual study.

The most common haplotypes in our population for which there was sufficient prevalence for analysis demonstrated no HLA haplotype association with cervical cancer. However, haplotypes of particular note are the HLA-A-B-DR haplotypes containing either HLA-B*07 or HLA-DRB*13 which were previously reported to alter cervical cancer risk. For those associated haplotypes, only one haplotype containing HLA-B*07 was demonstrated to increase risk of cervical neoplasia; however, the rarity of this and other haplotypes with HLA-B*07 precludes their explanation as underlying previously reported HLA-B*07 risk associations. Consistent decreased risks (albeit not statistically significant) were observed for haplotypes including HLA-DR*13. Because the inferred haplotypes with those alleles were also very rare, we believe that our results indicate that previous associations observed with HLA-DR*13 alleles are likely not due to linkage disequilibrium with other HLA alleles measured here. For cervical cancer research, this is an important concept, particularly for its implication in identifying HLA-binding motifs and in understanding presentation of foreign antigens such as HPV as they relate to HLA molecules. We conclude that

Table 3 Risk estimates[a] of cervical cancer with selected HLA-A-B and HLA-A-B-DR assigned haplotypes: combined results from Costa Rican, Eastern US and Portland studies

HLA haplotype		No. of cases (*n* 365)	No. of controls (*n* 681)	OR[a](95% CI)
A-B	01-07	13	24	1.1 (0.6–2.2)
	01-08	29	74	0.8 (0.5–1.3)
	02-07	37	63	1.2 (0.8–1.8)
	02-27	6	27	0.5 (0.2–1.2)
	02-35	22	40	0.9 (0.5–1.5)
	02-40	26	45	1.2 (0.7–1.9)
	02-44	50	91	1.2 (0.8–1.7)
	03-07	31	58	1.1 (0.7–1.8)
	29-44	17	26	1.2 (0.7–2.3)
A-B-DR[b]	01-08-03	14	22	1.1 (0.5–2.2)
	02-07-07	9	4	3.9 (1.2–13.0)
	02-15-13	0	9	0.3 (0.1–1.2)
	02-40-04	15	14	1.5 (0.7–3.1)
	02-44-13	1	14	0.6 (0.3–1.4)
	02-51-13	2	7	0.4 (0.1–1.3)
	03-07-15	32	13	0.9 (0.5–1.7)

[a]Adjusted for study.

[b]Includes HLA-DR*13 from Portland US study.

our previously reported allele associations are not likely to be attributable to linkage disequilibrium with other HLA alleles and underlying haplotypes.

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